Bull. Org. mond. Santé / *Bull. Wld Hlth Org.* 1968, 39, 917-924

International Standard for Anti-Canine-Distemper Serum *

DOREEN L. STEWART, C. NANCY HEBERT & IAN DAVIDSON

The Central Veterinary Laboratory, Weybridge, England, was requested by the WHO Expert Committee on Biological Standardization to obtain suitable material for an international standard for anti-canine-distemper serum and to arrange a collaborative assay. Seven laboratories in 6 countries assayed a batch of anti-canine-distemper serum against 3 test preparations. On the basis of the results obtained, the material has been established as the International Standard for Anti-Canine-Distemper Serum and the International Unit of Anti-Canine-Distemper Serum has been defined as the activity contained in 0.0897 mg of the International Standard.

The WHO Expert Committee on Biological Standardization of the World Health Organization (1964) noted that there was a need for an international standard for anti-canine-distemper serum. The Committee requested the Central Veterinary Laboratory, Weybridge, England, to obtain suitable material and to arrange a collaborative assay.

Glaxo Laboratories, Greenford, Middlesex, England, donated 4 litres of anti-canine-distemper serum prepared in a horse. The serum was distributed into ampoules in 1-ml amounts and freeze-dried. The ampoules were filled with dry nitrogen, then sealed and stored at −20°C.

INTERNATIONAL COLLABORATVE ASSAY

Seven laboratories in 6 countries took part in the assay. They are listed in Annex 1 and referred to in the text by arbitrary numbers which do not necessarily correspond to the order in which they are listed in the annex.

Participants were asked to titrate the proposed standard and 3 test preparations in parallel by serum-neutralization tests. The following test preparations, the identities of which were not disclosed to the participants, were supplied:

Preparation DA. A 1 : 2 dilution of the proposed international standard prepared by reconstituting the contents of each of several ampoules of the proposed international standard in 1-ml sterile distilled water, pooling the material, diluting 1 : 2 with M/100 phosphate buffer, pH 7.2, and redistributing the liquid in 5-ml amounts into bottles. Each participant received 1 bottle of DA.

Preparation DB. The American National Standard for anti-canine-distemper serum prepared in dogs. Several vials, each containing 10 ml of liquid serum, were obtained from the National Animal Disease Laboratory, Ames, Iowa, USA. One vial was sent to each participant.

Preparation DC. A commercial serum prepared in dogs for therapeutic use. Five-ml aliquots were dispensed into bottles from a serum pool and 1 bottle of liquid serum was sent to each participant.

Collaborators were asked to perform serum-neutralization tests by their own methods. Six laboratories titrated the materials in embryonated eggs by inoculation on to the chorioallantoic membrane (CAM). Laboratories 1, 2, 3, 5 and 7 used the dropped-membrane technique and laboratory 4 the Gorham (modified dropped-membrane) method. The main features of the egg titrations are summarized in Table 1.

Laboratory 6 performed titrations in 6-day-old primary dog kidney cultures in tubes. The antigen used was the Rockborn strain. Virus serum mixtures were left to neutralize for 1 hour at room temperature (20°C–25°C), after which time 0.2 ml of each mixture was inoculated into each of 5 tubes. The tubes were read after inoculation at 37°C for 10 days.

All laboratories used dilutions of serum and a constant amount of virus for each test. To each serum dilution was added an equal volume of virus suspension containing a calculated number of 50%

* From the Department of Biological Products and Standards, Central Veterinary Laboratory, Weybridge, Surrey, England.

TABLE 1

METHODS USED IN 7 LABORATORIES FOR ASSAYING THE PROPOSED INTERNATIONAL STANDARD OF ANTI-CANINE-DISTEMPER SERUM

	Laboratory No.						
	1	2	3	4	5	6	7
Strain of virus	Baker 0	Onderstepoort	Pitman-Moore	Onderstepoort	Onderstepoort	Pitman-Moore P-43	
Diluent	0.85 % saline + 5 % horse serum	Earle's saline + yeast + lactalbumin	Meat broth	Phosphate buffer + lactalbumin + sucrose	90 % Snyder's solution + 9 % Hank's BSS + 1 % bovine albumin fraction V	Nutrient broth (Difco)	
Age of eggs (days)	8–9	8	9	8	8	7	
Time and temperature of neutralization	1 ½ h, room temperature [a]	2 h; +4°C	1 h; room temperature [a]	2 h; +4°C	2 h; ice-bath	1 h; +4°C	
No. of eggs per dilution in final test	6	6	6–8	6	8	6	
Volume of mixture inoculated per egg (ml)	0.1	0.1	0.2	0.2	0.2	0.2	
Time and temperature of incubation of inoculated eggs	6 days; 37°C	6 days; 37°C	6 days; 35°C	6 days; 37°C	6 days; 37°C		5 days; 37.5°C

[a] Normally 20°C–25°C

egg-infective doses (EID_{50}) or 50% tissue-culture infective doses ($TCID_{50}$) obtained from preliminary antigen titrations. This number varied from one laboratory to another. Antigen titrations were performed simultaneously with the serum-neutralization tests to check that the actual number of EID_{50} or $TCID_{50}$ compared favourably with the calculated number.

STATISTICAL ANALYSIS OF RESULTS

A total of 20 assays submitted by the 7 laboratories was included in the analysis. In titrations performed by laboratory 6, the number of tubes infected out of the total number of tubes, and in all other titrations, the number of eggs which showed lesions out of the total number of eggs inoculated, was taken as the response. The dose was expressed throughout as the initial dilution of serum before the addition of an equal volume of virus. In all final assays, 2-fold or 4-fold dilutions of serum were used, giving a valid estimate of slope in all assays, which were therefore analysed by the method of probit analysis. The preliminary assays for laboratories 3, 5 and 7 were included with the final assays since 2-fold or 4-fold dilutions were used instead of 10-fold as in other preliminary titrations.

In this report, the titre of a serum is expressed as a PD_{50} value, that is, the dilution of serum which prevents infection of 50% of the membranes or tubes examined. PD_{50} estimates obtained in the analysis of individual assays by the probit method are given in Annex 2, and the 95% confidence limits are shown. The number of EID_{50} or $TCID_{50}$ of virus inoculated per egg or tube (with 95% confidence limits) are given in Annex 3.

Laboratory 1 tested 5 ampoules of the proposed standard in both assays. In the first, PD_{50} titres varied over a 3-fold range, but in the second, the titres of 4 ampoules were within 2% of each other, while the fifth ampoule was 1.7 times higher. Laboratory 6 tested 4 ampoules of the proposed standard in each assay. Titres of individual ampoules were less variable than those found by laboratory 1, although there was a 2-fold variation in the first assay, due to 1 ampoule having a higher titre.

Laboratories 1 and 3 included local preparations in their assays and PD_{50} values are shown in Annex 2.

In tests of this type, it is not unusual to find some eggs which do not react and in titrations performed by 2 laboratories there were isolated instances where a single negative membrane was found at a low serum concentration where 100% positive membranes might have been expected. However,

as similar negative membranes could have occurred at any point in the dose range without being detected, these results were included in the analysis. Their inclusion did not appreciably affect either PD_{50} or potency estimates.

Estimation of relative potency

Estimates of the potency of preparations DA, DB and DC relative to the proposed standard in the 20 individual assays are shown in Annex 4. In only one assay (assay 3, laboratory 5) was there a highly significant deviation from parallelism ($P < 0.01$). This was due to the abnormally low slope obtained for preparation DA, results for which were therefore excluded from the analysis. Weighted mean relative potencies for each laboratory are given in Table 2, together with their weights (the sum of the reciprocals of the individual variances).

TABLE 2
WEIGHTED MEAN RELATIVE POTENCIES FOR EACH LABORATORY

Laboratory	No. of assays	Relative potency [a]	Statistical weight	χ^2 test of homogeneity	
				χ^2	Significance
		Preparation DA			
1	2	0.636 (0.392–1.032)	87.2	0.42	0.70 > P > 0.50
2	3	0.530 (0.363–0.775)	141.4	1.91	0.50 > P > 0.30
3	3	0.496 (0.354–0.694)	179.6	0.87	0.70 > P > 0.50
4	2	0.320 (0.166–0.619)	47.0	0.56	0.50 > P > 0.30
5	4	0.661 (0.516–0.846)	333.7	0.68	0.90 > P > 0.80
6	2	0.449 (0.340–0.593)	261.9	0.59	0.50 > P > 0.30
7	3	0.855 (0.599–1.222)	160.0	5.53	0.10 > P > 0.05
		Preparation DB			
1	2	0.377 (0.225–0.632)	76.4	0.06	0.90 > P > 0.80
2	3	0.366 (0.250–0.536)	139.9	0.41	0.90 > P > 0.80
3	3	0.288 (0.164–0.317)	186.4	1.23	0.70 > P > 0.50
4	2	1.207 (0.603–2.417)	42.2	0.20	0.70 > P > 0.50
5	5	0.304 (0.245–0.379)	425.5	16.81	0.01 > P > 0.001
6	2	0.196 (0.146–0.262)	239.9	0.96	0.50 > P > 0.30
7	3	0.651 (0.457–0.928)	162.4	0.80	0.70 > P > 0.50
		Preparation DC			
1	2	0.769 (0.458–1.289)	76.3	3.42	0.10 > P > 0.05
2	3	1.060 (0.727–1.545)	143.4	0.51	0.80 > P > 0.70
3	3	0.575 (0.413–0.802)	184.5	1.38	0.70 > P >0.50
4	2	2.376 (1.213–4.652)	45.1	0.29	0.70 > P > 0.50
5	5	0.841 (0.677–1.044)	435.8	6.95	0.20 > P > 0.10
6	2	1.796 (1.359–2.373)	261.9	0.59	0.50 > P > 0.30
7	3	1.418 (0.972–2.070)	142.4	3.07	0.30 > P > 0.20
		Local preparations			
1	2	1.311 (0.779–2.205)	75.3	0.16	0.70 > P > 0.50
3	3	0.849 (0.606–1.190)	179.2	0.38	0.90 > P > 0.80

[a] 95 % confidence limits in parentheses.

TABLE 3

OVER-ALL POTENCY ESTIMATES

A. Weighted mean potencies

Preparation	Laboratories	No. of assays	Relative potency [a]	Statistical weight	χ^2 tests of homogeneity					
					Within laboratories			Between laboratories		
					χ^2	d.f.[b]	Significance	χ^2	d.f.[b]	Significance
DA	1–7	19	0.570 (0.500–0.649)	1 210.8	10.56	12	0.70 > P > 0.50	13.27	6	0.05 > P > 0.02
DB	1–3, 6	10	0.254 (0.212–0.303)	642.6	2.66	6	0.90 > P > 0.80	9.23	3	0.05 > P > 0.02
	1–3, 6, 7	13	0.307 (0.262–0.360)	805.0	3.46	8	0.95 > P > 0.90	30.95	4	P < 0.001
	1–4, 6, 7	15	0.328 (0.281–0.384)	847.2	3.66	9	0.95 > P > 0.90	45.14	5	P < 0.001
DC	1–7	20	1.042 (0.919–1.181)	1 289.4	16.21	13	0.30 > P > 0.20	40.35	6	P < 0.001

B. Unweighted mean potencies

Preparation	Laboratories	No. of assays	Relative potency [a]	Statistical weight
DA	1–7	19	0.553 (0.461–0.662)	716.7
DB	1–3, 5, 6	15	0.279 (0.227–0.342)	578.0
	1–3, 5–7	18	0.319 (0.254–0.401)	446.1
	1–7	20	0.364 (0.275–0.483)	293.9
DC	1–7	20	1.051 (0.802–1.378)	319.1

[a] 95 % confidence limits in parentheses.
[b] Degrees of freedom.

Table 3 shows the over-all weighted mean potencies for the 3 test preparations, and χ^2 tests of the significance of within- and between-laboratory variation. The over-all mean relative potencies calculated directly from the unweighted log potencies are also given.

Preparation DA. This was a 1 : 2 dilution of the proposed standard and the over-all mean potency for the 19 assays included was found to be 0.570 using weighted log potencies and 0.553 by the direct method. There was no significant variation either within or between laboratories (Tables 2 and 3) but individual assay results ranged from 0.25 to 1.48.

Preparation DB. Estimates of relative potencies obtained in individual assays ranged from 0.14 to 1.41. There was a highly significant variation between the 5 assays performed by laboratory 5, and the weighted mean potency shown in Table 2 (0.304) is not strictly valid, and cannot be included in an estimate of the over-all weighted mean potency. The mean for this laboratory calculated by the direct method is 0.278. Both values lie close to the over-all means for the collaborative assay.

There was a highly significant variation between laboratories, due almost entirely to the results obtained by laboratories 4 and 7. Potencies in both assays performed by laboratory 4 were exceptionally high. Over-all weighted mean potencies including and excluding these 2 laboratories are given in Table 3. Mean potencies calculated by the direct method, again including and excluding laboratories 4 and 7, but including laboratory 5, are also shown. Values of 0.36 and 0.28, respectively, were obtained by this method.

Preparation DC. There was again considerable variation between the relative potencies obtained at different laboratories, although there was no significant within-laboratory variation. However,

the over-all weighted mean potency (1.042) agreed closely with that obtained by the direct method (1.051). Potencies in individual assays ranged from 0.42 to 2.97.

Local preparations. The relative potencies of the local preparations included by laboratories 1 and 3 are given in Annex 4 and Table 2. There was no significant variation between assays at either laboratory, and neither preparation differed significantly in potency from the proposed standard.

Alternative method of analysis

In routine titrations of this type, PD_{50} titres are normally evaluated by a method involving simpler calculations than those required in the method of probit analysis. Estimates by the Spearman–Kärber method, which is considered the most satisfactory alternative, were therefore calculated in addition to those found by the probit method, and relative potencies were evaluated from the ratio of the PD_{50} titres, the variance of each log potency being the sum of the variances of the 2 PD_{50} values compared. Results obtained by this method were very similar to those evaluated by the probit method and have not, therefore, been included.

CONCLUSIONS

It is generally accepted that titrations by the CAM method are subject to many sources of error, but the precision of these assays was reasonably high and results were usually consistent. The potencies obtained for preparation DA, which was a 1 : 2 dilution of the proposed international standard, show that, although individual estimates varied over a 3-fold range, the over-all mean for the 10 assays was close to the known potency of 0.5. Moreover, when the range of PD_{50} values for each preparation is compared with the corresponding range of relative potencies, it is found that the standard considerably reduces the variability for all preparations.

The WHO Expert Committee on Biological Standardization (1968) established the proposed standard as the International Standard for Anti-Canine-Distemper Serum. The unitage of the International Standard for Anti-Canine-Distemper Serum has been defined so that each ampoule contains 1000 International Units. The International Unit of Anti-Canine-Distemper Serum is thus defined as the activity contained in 0.0897 mg of the International Standard of Anti-Canine-Distemper Serum.

RÉSUMÉ

Le Comité OMS d'experts de la Standardisation biologique, notant qu'il serait nécessaire d'établir un étalon international de sérum anti-maladie du jeune chien (maladie de Carré), avait demandé au Central Veterinary Laboratory de Weybridge, Angleterre, de se procurer du matériel approprié et d'organiser un titrage comparatif.

Un lot de sérum anti-maladie du jeune chien a pu être obtenu et a fait l'objet, en même temps que 3 autres préparations, d'un titrage par épreuves de séroneutralisation dans 7 laboratoires de six pays.

Sur la base des résultats obtenus, le Comité OMS d'experts de la Standardisation biologique a constitué l'étalon proposé en étalon international de sérum anti-maladie du jeune chien (maladie de Carré). Avec l'accord des participants au titrage comparatif, il a défini l'unité internationale de sérum anti-maladie du jeune chien (maladie de Carré) comme l'activité de 0,0897 mg de l'étalon international.

REFERENCES

WHO Expert Committee on Biological Standardization (1964) *Wld Hlth Org. techn. Rep. Ser.*, **274**, 23

WHO Expert Committee on Biological Standardization (1968) *Wld Hlth Org. techn. Rep. Ser.*, **384**, 18

Annex 1

PARTICIPANTS IN THE COLLABORATIVE ASSAY

AUSTRALIA

Mr A. G. Mathews
Commonwealth Serum Laboratories
45 Poplar Road
Parkville
Victoria, 3052

CANADA

Dr K. F. Lawson
Connaught Laboratories
1755 Steeles Avenue West
Willowdale
Ontario

FEDERAL REPUBLIC OF GERMANY

Dr K. Dräger
Behringwerke AG
Marburg-Lahn 1

FRANCE [1]

Professor P. Goret
Ecole Vétérinaire d'Alfort
7 Avenue du Général de Gaulle
94 Alfort

Dr Jean Terré
Institut français de la Fièvre aphteuse
254 Rue Marcel Mérieux
Lyon

UNITED KINGDOM OF GREAT BRITAIN AND NORTHERN IRELAND

Dr Joan M. Barnes
Biological Control Unit
Glaxo Laboratories Ltd.
Greenford
Middlesex

Miss D. L. Stewart
Ministry of Agriculture, Fisheries and Food
Central Veterinary Laboratory
New Haw
Weybridge
Surrey

UNITED STATES OF AMERICA

Dr J. A. Gourlay
National Animal Disease Laboratory
P.O. Box 905
Ames
Iowa, 50010

[1] The 2 laboratories undertook the collaborative assay jointly.

Annex 2

PD_{50} RECIPROCAL TITRES OF PROPOSED STANDARD AND TEST PREPARATIONS

Laboratory	Assay	Preparation	PD_{50} [a]
1	1	D1	492 (244–993)
		D2	358 (177–721)
		D3	556 (275–1 125)
		D4	216 (90–517)
		D5	184 (88–384)
		D (1–5)	352 (253–488)
		DA	189 (133–268)
		DB	145 (88–236)
		DC	148 (101–218)
		LP	512 (369–709)
	2	D1	337 (182–623)
		D2	200 (111–361)
		D3	198 (110–358)
		D4	202 (112–365)
		D5	198 (110–358)
		D (1–5)	216 (166–283)
		DA	160 (119–216)
		DB	78 (59–102)
		DC	247 (189–324)
		LP	255 (194–333)
2	1	D	231 (146–366)
		DA	84 (53–132)
		DB	102 (65–161)
		DC	291 (183–462)
	2	D	188 (123–286)
		DA	102 (67–155)
		DB	62 (41– 96)
		DC	168 (110–257)
	3	D	179 (116–277)
		DA	127 (82–197)
		DB	62 (40– 95)
		DC	193 (125–299)
3	1	D	760 (397–1 457)
		DA	257 (134–491)
		DB	128 (67–244)
		DC	409 (214–781)
		LP	594 (310–1 139)
	2	D	586 (392–877)
		DA	330 (222–490)
		DB	116 (78–173)
		DC	262 (176–389)
		LP	581 (390–866)
	3	D	508 (369–699)
		DA	256 (186–352)
		DB	138 (100–190)
		DC	350 (255–482)
		LP	403 (292–554)
4	1	D	66 (36–122)
		DA	28 (15– 52)
		DB	68 (35–132)
		DC	190 (98–370)
4	2	D	60 (32–110)
		DA	15 (8– 27)
		DB	84 (41–170)
		DC	118 (65–215)
5	1	D	128 (73–224)
		DA	93 (53–162)
		DB	17 (10– 31)
		DC	76 (43–133)
	2	D	101 (73–139)
		DA	57 (41– 78)
		DB	44 (32– 60)
		DC	58 (42– 79)
	3	D	98 (71–135)
		DA	75 (55–104)
		DB	30 (22– 42)
		DC	75 (54–104)
	4	D	81 (63–103)
		DA	58 (45– 74)
		DB	20 (15– 25)
		DC	78 (61–100)
	5	D	66 (46– 93)
		DA	45 (32– 64)
		DB	24 (17– 34)
		DC	85 (60–120)
6	1	D1	60 (41– 88)
		D2	60 (42– 86)
		D3	52 (31– 87)
		D4	104 (66–164)
		D (1–4)	66 (55– 80)
		DA	34 (28– 41)
		DB	17 (12– 23)
		DC	137 (113–166)
	2	D1	69 (48– 98)
		D2	60 (42– 86)
		D3	60 (42– 86)
		D4	69 (48– 98)
		D (1–4)	64 (55– 74)
		DA	26 (23– 31)
		DB	11 (10– 13)
		DC	105 (91–123)
7	1	D	188 (125–283)
		DA	103 (69–156)
		DB	91 (61–137)
		DC	208 (138–312)
	2	D	135 (96–190)
		DA	200 (142–281)
		DB	100 (71–141)
		DC	401 (285–563)
	3	D	149 (105–211)
		DA	100 (70–142)
		DB	100 (70–142)
		DC	200 (141–284)

[a] 95 % confidence limits in parentheses.

Annex 3

NUMBER OF EID_{50} OR $TCID_{50}$ OF VIRUS USED [a]

Laboratory	Assay	No. of EID_{50} [b]	Laboratory	Assay	No. of EID_{50} or $TCID_{50}$ [b]
1	1	42 (19– 93)	5	1	291 (94–904)
	2	160 (42–611)		2	164 (90–301)
				3	116 (49–275)
2	1	316		4	308 (138–685)
	2	178 (31–1 030)		5	197 (108–360)
	3	85 (23–320)	6	1	181 (110–299)
3	1	33 (24– 45)		2	416 (263–658)
	2	60 (43– 83)	7	1	369 (86–1 580)
	3	38 (28– 51)		2	681 (242–1 920)
4	1	174 (19–1 580)		3	681 (242–1 920)
	2	1 240 (210–7 310)			

[a] Calculated for 0.1 ml virus–serum mixture for laboratories 1 and 2, and for 0.2 ml for laboratories 3–7.
[b] 95 % confidence limits in parentheses.

Annex 4

POTENCY OF TEST PREPARATIONS DA, DB AND DC AND OF LOCAL PREPARATIONS RELATIVE TO THE PROPOSED STANDARD

Laboratory	Assay	Preparation DA [a]	Preparation DB [a]	Preparation DC [a]
1	1	0.538 (0.266–1.083)	0.412 (0.170–0.998)	0.422 (0.184–0.946)
	2	0.740 (0.384–1.458)	0.360 (0.188–0.678)	1.143 (0.584–2.214)
2	1	0.363 (0.177–0.727)	0.442 (0.219–0.877)	1.257 (0.639–2.489)
	2	0.542 (0.290–1.021)	0.332 (0.172–0.619)	0.896 (0.477–1.702)
	3	0.711 (0.373–1.353)	0.343 (0.178–0.657)	1.080 (0.567–2.057)
3	1	0.338 (0.135–0.834)	0.168 (0.069–0.404)	0.538 (0.215–1.307)
	2	0.562 (0.320–1.001)	0.198 (0.114–0.352)	0.447 (0.254–0.794)
	3	0.504 (0.316–0.807)	0.271 (0.172–0.431)	0.690 (0.437–1.095)
4	1	0.421 (0.170–1.187)	1.025 (0.366–2.692)	2.878 (1.192–8.202)
	2	0.254 (0.108–0.644)	1.407 (0.515–3.580)	1.983 (0.842–5.492)
5	1	0.723 (0.316–1.647)	0.136 (0.058–0.326)	0.593 (0.266–1.322)
	2	0.560 (0.346–0.947)	0.432 (0.270–0.689)	0.571 (0.357–0.905)
	3	—	0.311 (0.193–0.505)	0.768 (0.478–1.250)
	4	0.718 (0.488–1.051)	0.245 (0.168–0.365)	0.971 (0.665–1.423)
	5	0.666 (0.396–1.105)	0.372 (0.228–0.614)	1.298 (0.794–2.161)
6	1	0.515 (0.329–0.809)	0.259 (0.157–0.398)	2.061 (1.316–3.235)
	2	0.412 (0.288–0.588)	0.177 (0.124–0.252)	1.647 (1.154–2.352)
7	1	0.550 (0.243–0.992)	0.485 (0.197–0.892)	1.106 (0.617–2.079)
	2	1.480 (0.914–2.952)	0.740 (0.396–1.190)	2.966 (1.543–10.015)
	3	0.672 (0.341–1.099)	0.672 (0.341–1.100)	1.344 (0.824–2.551)

Potency of local preparations

Laboratory	Assay	Relative potency [a]
1	1	1.456 (0.709–3.050)
	2	1.176 (0.557–2.457)
3	1	0.781 (0.335–1.829)
	2	0.992 (0.552–1.832)
	3	0.793 (0.499–1.265)

[a] 95 % confidence limits in parentheses.